Effects of Sucrose Esters of Fatty Acids on Flat Sour Spoilage by Obligate Anaerobes *

Akihiko NAKAYAMA, Junko SONOBE and Rieko SHINYA

The effects of two commercial emulsifiers (sucrose esters of fatty acids with C_{16} and C_{18} fatty acid moieties) on the growth, germination and/or outgrowth of six strains of the causative bacteria of O. A. flat sour spoilage were studied.

In the TSiF medium, both emulsifiers at concentrations higher than 100 ppm inhibited the growth of all six strains. For germination and/or outgrowth, the minimal inhibitory concentration (MIC) of one of the emulsifiers was 10 ppm and that of the other was 10 to 100 ppm depending on the strain.

In the coffee medium, the highest MIC of both emulsifiers for growth, germination and/or outgrowth was 300 ppm. In the *shiruko* medium, the highest MIC of one of the emulsifiers for growth was 5000 ppm and that of the other was 6000 ppm. The highest MIC of both emulsifiers for germination and/or outgrowth was 5000 ppm.

It is concluded that the sucrose esters can prevent O. A. flat sour spoilage at 300 ppm in canned coffee and at 5000 ppm in canned *shiruko*. It is suggested that the addition of the sucrose esters would effectively prevent the spoilage of canned drinks kept hot in vending machines in Japan.

Introduction

In previous papers[1)~4)], we showed that a new type of flat sour spoilage (O. A. flat sour spoilage) of canned drinks, kept heated in vending machines in Japan, is caused by thermophilic obligate anaerobes, in contrast to the flat sour spoilage known to be caused by the thermophilic facultative anaerobes, *Bacillus stearothermophilus* or *Bacillus coagulans*[5)]. We also reported that the heat resistance of the causative bacteria is so high that the conventional thermal processes are insufficient to sterilize the canned drinks[2)~4)]. As a countermeasure to the spoilage, prior UV irradiation of the dissolved sugar, which is probably the source of the causative bacteria, was recommended[6),7)].

However, the possibility remains that the causative bacteria may also arise from ingredients other than sugar, and a more reliable means for preventing the spoilage is desirable. In this paper we describe the preventive effects on O. A. flat sour spoilage in canned drinks, of sucrose esters of fatty acids, which have been used in canned coffee as an emulsifier and are known to inhibit the growth of microorganisms[8)~16)].

Materials and Methods

Microorganisms

The bacteria tested were the six isolates (Strain Nos. 24-1, 13-1, 26-11, 27-8, 28-3 and 28-4) which have been found in our laboratory to be the causative bacteria of O. A. flat sour spoilage[2)~4)]. Strain Nos. 24-1, 13-1, 26-11 and 27-8 were isolated from spoiled canned coffee samples, and Strain Nos. 28-3 and 28-4 were

* A New Type of Flat Sour Spoilage —VI

注 本論文は日本食品衛生学会の好意により、食品衛生学雑誌・第23巻第1号掲載論文を転載したものである。

isolated from spoiled canned *shiruko* (a sweet adzuki bean drink) samples.

Sucrose esters of fatty acids

The sucrose esters of fatty acids used were two kinds of commercial emulsifier, P-1570 and S-1570 (Ryoto Co., Ltd.), with C_{16} and C_{18} fatty acid moieties. The compositions of the fatty acids and sucrose esters of the emulsifiers are shown in Table 1.

Table 1. Compositions of Fatty Acids and Esters of the Emulsifiers P-1570 and S-1570*

Sucrose ester	Fatty acid moieties		Esters	
	Palmitic	Stearic	Mono	Di & tri
P-1570	70%	30%	70%	30%
S-1570	30	70	70	30

* Approximate concentrations according to Ryoto Co., Ltd., Tokyo, Japan

Inhibition tests of sucrose esters of fatty acids in TSiF medium[2]

Because the emulsifiers do not give clear solutions when the concentrations are higher than 20~30 ppm, detection of the growth of bacteria by turbidity measurements is not suitable. The TSiF medium, which is blackened by the reduction of sulfite with the growth of the bacteria, was used for the inhibition tests. The concentrations of the emulsifiers ranged from 0 to 1000 ppm for the tests of the effects on growth and from 0 to 100 ppm for the effects on the germination and/or outgrowth.

For the former tests, 0.2 ml aliquots of the mTGC medium[17] incubated at 55°C for 10 days with the bacteria were inoculated into 10 ml of the TSiF media containing the emulsifiers at various concentrations. All these media were sealed with sterile liquid paraffin and incubated at 55°C for 15 days. The growth of the bacteria was observed by following the blackening of the media. The experiments were run in duplicate.

For the latter tests, spore suspensions of the strains prepared by the method described previously[2] were used. The spore concentrations of the strains were as follows: Strain No. 24-1, 2.3×10^6/ml; Strain No. 13-1, 6.4×10^4/ml; Strain No. 26-11, 1.0×10^5/ml; Strain No. 27-8, 6.4×10^5/ml; Strain No. 28-3, 1.0×10^4/ml; Strain No. 28-4, 9.5×10^4/ml. The inoculation and the incubation were carried out in the same manner as in the former tests.

Inhibition tests of sucrose esters of fatty acids in coffee and shiruko media

The formulae of coffee and *shiruko* media are shown in Table 2.

Table 2. Formulae of Coffee and *shiruko* media

Coffee medium

Ingredients	Concentration
Coffee extract	85 %(v/v)
Cow's milk	15 %(v/v)
Sugar	8 %(w/v)
Skim milk	2 %(w/v)
$NaHCO_3$	0.06%(w/v)
pH: 6.3~6.5	

Shiruko medium

Ingredients	Concentration
Canned *yudeadzuki*	45 %(w/v)
NaCl	0.1 %(w/v)
pH: 6.2	
Brix: 18%	

Roasted coffee beans (Colombia, Ueshima Coffee Co., Ltd.), cow's milk, skim milk, granulated sugar and sodium bicarbonate (special grade) were commercially purchased.

To obtain coffee extract, 25 g of roasted coffee beans was ground and extracted with 500 ml of deionized water in an electric coffee maker. To remove bacteria from the sugar, especially bacteria similar to the strains used, granulated sugar was dissolved in an appropriate amount of deionized water, and the solution was filtered through Millipore filters (GSWP 047 S0). The filtrate was concentrated under a vacuum. The concentrations of the emulsifiers added to the coffee medium ranged from 0 to 500 ppm at intervals of 100 ppm.

Canned *yudeadzuki* (traditional Japanese boiled and sweetened adzuki bean product) and sodium chloride (special grade) were commercially purchased.

To prepare the *shiruko* medium, the commercial canned *yudeadzuki* was diluted with deionized water (45% w/v). The mixture was homogenized and sodium chloride (0.1% w/v) was added to the homogenate. To this *shiruko* medium, the emulsifiers were added at concentrations up to 6000 ppm at intervals of 1000 ppm.

Ten ml aliquots of all these media were distributed into test tubes, autoclaved, and sealed immediately with sterile liquid paraffin.

The cultures (mTGC media) and spore suspensions of the strains were inoculated in the same manner for the tests with TSiF media, except that five coffee and five *shiruko* media were used for each concentration of the emulsifier instead of duplicate experiments as in the case of TSiF medium. The spore concentrations of the strains were as follows: Strain No. 24-1, 4.9×10^6/ml; Strain No. 13-1, 2.4×10^4/ml; Strain No. 26-11, 1.0×10^4/ml; Strain No. 27-8, 7.9×10^3/ml; Strain No. 28-3, 1.1×10^2/ml; Strain No. 28-4, 3.5×10^6/ml. All these media were incubated at 55°C for 30 days and their pH values were measured to detect spoilage.

As controls, 30 coffee media and 10 *shiruko* media containing the emulsifiers at each concentration were treated in the same way but without the inoculation.

Results

Inhibitory effects of sucrose esters of fatty acids in TSiF medium

Table 3 shows the effects of the emulsifiers (P-1570 and S-1570) on the growth of the six strains. The media containing 100 ppm or higher concentrations of both emulsifiers were not blackened by any of the strains.

Table 4 shows the effects of the emulsifiers on the germination and/or outgrowth of the spores of the six strains. Strain Nos. 13-1 and 26-11 did not blacken the media containing 10 ppm or higher concentrations of P-1570, and the other strains did not blacken the media containing 100 ppm or more of the emulsifier. On the other hand, the media containing 10 ppm or more of S-1570 were not blackened by any of the strains tested.

Inhibitory effects of sucrose esters of fatty acids in coffee medium

To confirm their sterility, the thirty media containing different concentrations of the emulsifiers were incubated without inoculation of the strains. It was found that after 30 days at 55°C the pH values of the media ranged from 5.9 to 6.1. No spoilage was observed among the media.

The effects of the emulsifiers on the growth, germination and/or outgrowth of all six strains in the coffee medium are shown in Fig. 1. The pH values of spoiled media are given in the figures and those of normal ones in the figure legend. The pH values of spoiled media were lower than those of normal ones. The maximum drop of pH value was about 1.0 unit.

Strain No. 24-1 did not spoil the coffee media when the concentration of the emulsifiers was higher than 300 ppm in both experiments, with the cultures (mTGC media) (A) and the spore suspensions (B).

Strain No. 13-1 did not spoil the coffee media containing 200 ppm or higher concentration of P-1570 and those containing 300 ppm or higher concentration of S-1570 in both experiments (A and B).

Strain No. 26-11 did not spoil the coffee media containing 200 ppm or higher concentration of P-1570 in experiment A. However, in experiment B with P-1570 and both experiments A and B with S-1570, inhibitory effects were observed with 300 ppm or more of the emulsifiers.

Strain No. 27-8 did not spoil the coffee media containing 300 ppm or higher concentration of either emulsifier in all the experiments.

Strain No. 28-3 did not spoil the coffee media containing 200 ppm or higher concentration of P-1570 in experiment B. However, in experiment A with P-1570 and both experiments A and B with S-1570, inhibitory effects were observed with 300 ppm or more of the emulsifiers.

Strain No. 28-4 did not spoil the coffee media containing 300 ppm or higher concentration of either emulsifier in all the experiments.

Table 3. Effects of the Sucrose Esters of Fatty Acids on the Growth of the Strains in the TSiF Medium*[1]

Sucrose ester	Concn. of sucrose ester	Strain No.					
		24-1	13-1	26-11	27-8	28-3	28-4
P-1570	0 ppm	+, +*[2]	+, +*[2]	+, +*[2]	+, +*[2]	+, +*[2]	+, +*[2]
	0.01	+, +	+, +	+, +	+, +	+, +	+, +
	0.1	+, +	+, +	+, +	+, +	+, +	+, +
	1.0	+, +	+, +	+, +	+, +	+, +	+, +
	10	+, +	+, +	+, +	+, +	+, +	+, +
	100	−, −	−, −	−, −	−, −	−, −	−, −
	200	−, −	−, −	−, −	−, −	−, −	−, −
	300	−, −	−, −	−, −	−, −	−, −	−, −
	500	−, −	−, −	−, −	−, −	−, −	−, −
	1000	−, −	−, −	−, −	−, −	−, −	−, −
S-1570	0	+, +	+, +	+, +	+, +	+, +	+, +
	0.01	+, +	+, +	+, +	+, +	+, +	+, +
	0.1	+, +	+, +	+, +	+, +	+, +	+, +
	1.0	+, +	+, +	+, +	+, +	+, +	+, +
	10	+, +	+, +	+, +	+, +	+, +	+, +
	100	−, −	−, −	−, −	−, −	−, −	−, −
	200	−, −	−, −	−, −	−, −	−, −	−, −
	300	−, −	−, −	−, −	−, −	−, −	−, −
	500	−, −	−, −	−, −	−, −	−, −	−, −
	1000	−, −	−, −	−, −	−, −	−, −	−, −

*[1] Duplicate experiments

*[2] Blackening of the medium was observed, +: blackened, −: not blackened

Table 4. Effects of the Sucrose Esters of Fatty Acids on the Germination and/or Outgrowth of the Strains in the TSiF Medium*

Sucrose ester	Concn. of sucrose ester	Strain No.					
		24-1	13-1	26-11	27-8	28-3	28-4
P-1570	0 ppm	+, +	+, +	+, +	+, +	+, +	+, +
	0.01	+, +	+, +	+, +	+, +	+, +	+, +
	0.1	+, +	+, +	+, +	+, +	+, +	+, +
	1.0	+, +	+, +	+, +	+, +	+, +	+, +
	10	+, +	−, −	−, −	+, +	+, +	+, +
	100	−, −	−, −	−, −	−, −	−, −	−, −
S-1570	0	+, +	+, +	+, +	+, +	+, +	+, +
	0.01	+, +	+, +	+, +	+, +	+, +	+, +
	0.1	+, +	+, +	+, +	+, +	+, +	+, +
	1.0	+, +	+, +	+, +	+, +	+, +	+, +
	10	−, −	−, −	−, −	−, −	−, −	−, −
	100	−, −	−, −	−, −	−, −	−, −	−, −

* Symbols are the same as in Table 3.

Inhibitory effects of sucrose esters of fatty acids in the shiruko medium

To confirm their sterility, the ten media containing each concentration of the emulsifiers were incubated without inoculation of the strains. It was found that after 30 days at 55°C the pH values of the media ranged from 5.4 to 5.7. No spoilage was observed among the media.

The effects of the emulsifiers on the growth,

germination and/or outgrowth of the three strains in the *shiruko* medium are shown in Fig. 2. The pH values of spoiled media are given in the figures and those of normal ones in the figure legend. The pH values of spoiled media were lower than those of normal ones. The maximum drop of pH values was about 1.0 unit.

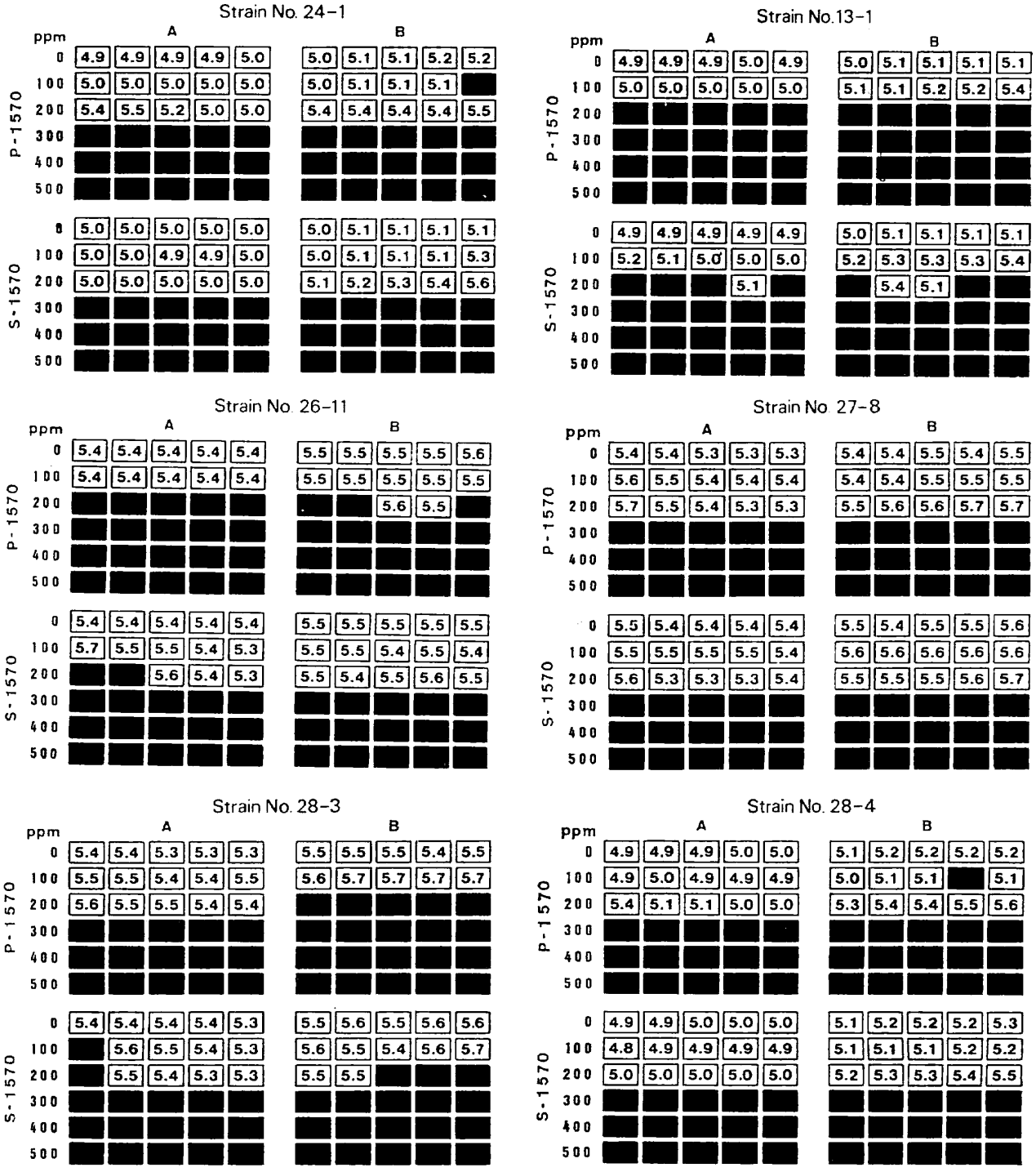

Fig. 1. Effects of the sucrose esters of fatty acids in the coffee medium on the growth, germination and/or outgrowth of the isolated strains

A: Vegetative cells inoculated, B: Spores inoculated

□: Spoiled (numerals show pH values), ■: Not spoiled (pH: 5.9~6.1)

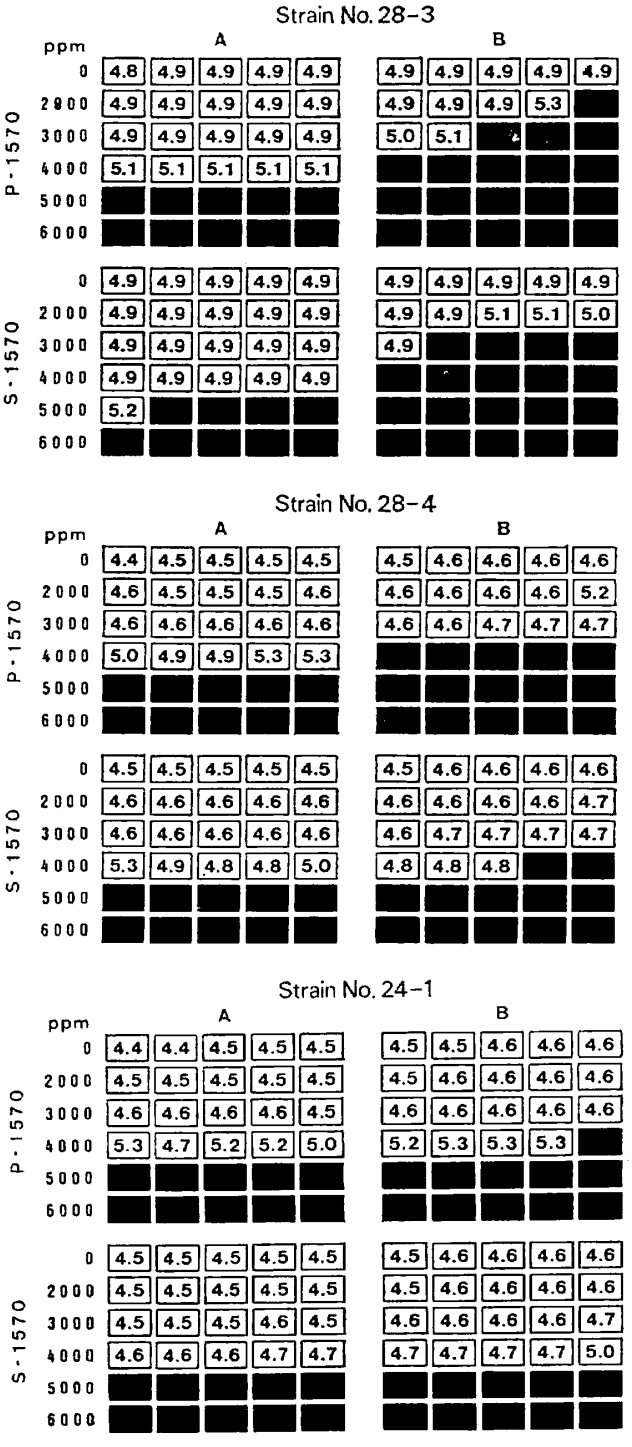

Fig. 2. Effects of the sucrose esters of fatty acids in the *shiruko* medium on the growth, germination and/or outgrowth of the isolated strains
A: Vegetative cells inoculated, B: Spores inoculated
□: Spoiled (numerals show pH values)
■: Not spoiled (pH: 5.4~5.6)

Strain No. 28-3 did not spoil the *shiruko* media containing 4000 ppm or higher concentration of either emulsifier in experiment B. However, in experiment A, inhibitions were observed with 5000 ppm or more of P-1570, and with 6000 ppm or more of S-1570.

Strain No. 28-4 did not spoil the *shiruko* media containing 4000 ppm or higher concentration of P-1570 in experiment B. In experiment A with P-1570 and S-1570 and experiment B with S-1570, inhibitions were observed with 5000 ppm or more of both emulsifiers.

Strain No. 24-1 did not spoil the *shiruko* media containing 5000 ppm or higher concentration of either emulsifier in all the experiments.

Discussion

The sucrose esters of fatty acids which have already been reported to have inhibitory effects on the growth of microorganisms are sucrose laurate[8),10)~13)], caprylate[12),13),16)], caprate[11),12),16)], myristate[11)], palmitate[9),11)], olaidate[11)], oleate[11)], linoleate[11)], etc. In these reports, the minimal inhibitory concentrations of these sucrose esters ranged from approximately 30 to 1000 ppm. According to Haenel and Müller-Beuthow, the minimal inhibitory concentrations of sucrose palmitate on some clostridia were 100 ppm[9)]. Conley and Kabara reported that the minimal inhibitory concentrations of sucrose palmitate on seven strains of Gram-positive bacteria were approximately 30 to 500 ppm[11)]. In this study, we found that sucrose palmitate-stearate affects the growth of the bacteria causing O. A. flat sour spoilage at the minimal inhibitory concentration of 100 ppm (Table 3), which falls in the ranges described above.

In the case of the inhibition tests in the TSiF medium, the growth of the strains was inhibited by 100 ppm of both emulsifiers. However, germination and/or outgrowth were inhibited by 10 or 100 ppm of P-1570, and by 10 ppm of S-1570 (Tables 3 and 4). These results show that the effects of the emulsifiers on the germination and/or outgrowth are greater than on the growth. It was found that P-1570 and S-1570 have equal effects on growth, but S-1570 is more effective on germination and/or outgrowth than P-1570.

In the case of inhibition tests with foods, a comparison of the effects of the emulsifiers on growth and on germination and/or outgrowth indicates that the latter are more sensitive than the former to the emulsifiers, except for Strain No. 26-11 (Figs. 1 and 2). These results agree with the results obtained in the TSiF medium (Tables 3 and 4).

A comparison of the effects of P-1570 and S-1570 suggests that the former is more effective, in contrast to the results obtained with the TSiF medium (Table 4). The reason for this difference is not known.

Strain Nos. 28-3 and 28-4, isolated from the canned *shiruko* samples, spoiled the coffee medium and the spoilage was inhibited by the emulsifiers in the same manner as with the four strains isolated from the canned coffee samples (Fig. 1.). Conversely, Strain No. 24-1, isolated from the canned coffee samples, spoiled the *shiruko* medium and the spoilage was inhibited by the emulsifiers in the same manner as with the two strains isolated from the canned *shiruko* sample (Fig. 2). These results further support our previous conclusion that O. A. flat sour spoilage of canned coffee and canned *shiruko* are caused by the same obligate anaerobes[4].

The lowest concentrations of the sucrose esters required to inhibit growth, germination and/or outgrowth in the TSiF, coffee and *shiruko* media increased markedly in that order. According to Bourne et al., sucrose monostearate forms an insoluble complex with starches[19]. Kato and Shibasaki reported that the antimicrobial activities of sucrose dicaprylate are influenced by some food constituents[12]. In view of these findings, it is considered that the results obtained here are due to loss of the inhibitory activities of the sucrose esters due to interaction with food constituents. Thus, especially in the case of the *shiruko* medium, the high concentration of starch in the adzuki beans means that a high concentration of the sucrose esters is required for the inhibition.

From all these results, it is concluded that the sucrose esters prevent O. A. flat sour spoilage at 300 ppm in canned coffee and at 5000 ppm in canned *shiruko*. Therefore, it is considered that the addition of the sucrose esters would effectively prevent the O. A. flat sour spoilage of canned drinks which are kept hot in vending machines in Japan.

In accord with this view, Japanese packers have informed the authors that the practical rates of flat sour spoilage among canned drinks appear to have decreased after the addition of emulsifiers.

Acknowledgement

The authors wish to express their thanks to Prof. Hajime Kadota, Faculty of Agriculture, Kyoto University, for many helpful discussions and suggestions throughout this study. Thanks are also due to Prof. Shigeru Otsuka, Toyo Junior College of Food Technology, for many discussions, and to Mr. Mikio Tsuruta, Ryoto Co., Ltd., for providing us with the samples of sucrose esters.

A part of this paper was presented at the annual meeting of the Japan. Soc. Sci. Fish. on Oct. 19, 1980, in Fukuoka.

References

1) Nakayama, A., Samo, S., Ikegami, Y.: Bull. Japan. Soc. Sci. Fish., **43**, 899 (1977).
2) Nakayama, A., Samo, S.: *ibid.*, **46**, 1117～1123 (1980).
3) Nakayama, A., Shinya, R.: J. Food Hyg. Soc. Japan, **22**, 30～36 (1981).
4) Nakayama, A., Shinya, R.: *ibid.*, **22**, 37～41 (1981).
5) Fields, M. L.: "Advances in Food Research" ed. by Chichester, C. O., Mrak, E. M., Stewart, G. F., Vol. 18, p. 163～217 (1970), Academic Press, New York.
6) Nakayama, A., Shinya, R.: J. Food Hyg. Soc. Japan. **22**, 415～420 (1981).
7) Nakayama, A., Shinya, R.: *ibid.*, **22**, 421～424 (1981).
8) Tani, Y., Yanagi, G., Ishida, M., Fukui, S.: J. Ferment. Technol., **39**, 92～98 (1961).
9) Haenel, H., Müller-Beuthow, W.: Nahrung, **10**, 23～29 (1966).
10) Kato, A., Arima, K.: Biochem. Biophys. Res. Commun., **42**, 596～601 (1971).
11) Conley, A. J., Kabara, J. J.: Antimicrob. Agents Chemother., **4**, 501～506 (1973).
12) Kato, N., Shibasaki, I.: J. Ferment. Technol., **53**, 793～801 (1975).
13) Kato, N., Shibasaki, I.: J. Antibact. Antifung. Agents, **3**, 355～361 (1975).
14) Shibasaki, I.: J. Ferment. Technol., **57**, 164～

176 (1979).
15) Shibasaki, I., Kato, N.: "The Pharmacological Effect of Lipid" ed. by Kabara, J. J., p. 15～24 (1979), American Oil Chemists' Society, Champaign, Illinois.
16) Beuchat, L. R.: Appl. Environ. Microbiol., **39**, 1178～1182 (1980).
17) Sakazaki, R.: Bacterial Culture Media, Part I, Kindai Shuppan, Tokyo, 1978, p. 277～278.
18) Bourne, E. J., Tiffin, A. I., Weigel, H.: J. Sci. Food Agric., **11**, 101～109 (1960).